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IN THE UNITED STATES AND TRADEMARK OFFICE

IN RE Application of

Josée Hamel, et al.

Serial No : 09/471,255 ✓

Filed : December 23, 1999

NOVEL STREPTOCOCCUS ANTIGENS

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DECLARATION OF DR. JOSÉE HAMEL

I, Josée Hamel, declare that:

1. I am a co-inventor of the invention of the subject Patent Application No. 09/471,255, entitled "*Novel Streptococcus Antigens*."
2. I am a citizen of Canada, residing at 2401 Maritain, Sillery, Quebec, G1T 1N6.
3. I am Director of the Immunology section at Vaccine Research Unit (Unité de Recherche en Vaccinologie) at the Laval University Research Center (Centre Hospitalier De l'Université Laval) and a professor at Laval University in the Medical Biology Department, Faculty of Medicine.
4. I have read the outstanding Official Action, mailed December 7, 2001. It is my understanding that the Examiner has rejected claims 16-20, which are directed to polypeptides having the specified sequence or polypeptides having at least 99% sequence similarity to the specified sequences, under 35 U.S.C. § 101. The Examiner asserts that no specific, credible and substantial utility for SEQ ID NO. 2 fragments or polypeptides that share amino acid sequence with SEQ ID NO. 2 or an epitope with SEQ ID NO. 2 has been disclosed.

I respectfully disagree with the Examiner's conclusion.

We isolated the novel polynucleotides disclosed and claimed in the present application by immunoscreening of a genomic expression library of *Streptococcus pneumoniae*, which allowed us to isolate genes and identify immunogenic proteins. In order to isolate the genes from a variety of *S. pneumoniae* we had to design sophisticated molecular tools. We designed sets of DNA probes that specifically detected the genes of interest in a variety of genetically unrelated *S. pneumoniae* isolates and generated monoclonal antibodies that were specific for selected regions of the polypeptides. Prior to our reported results, no one had been able to identify the full-length BVH-3 gene, although others had tried and failed.

We sequenced the isolated genes and on the basis of the nucleotide sequence, open reading frames, and predicted amino acid sequences using classical algorithms such as SpScan, determined that the encoded polypeptides had structural features characteristic of surface proteins. However, it was not obvious from the structural characteristics of the polypeptides whether the polypeptides and fragments of the polypeptides were immunogenic, and if so, which portions of the polypeptides confer protection. Moreover, it was not known at the time the polynucleotides were isolated whether or not these isolated genes were present in all species of *Streptococcus* and whether or not the genes and the encoded polypeptides were highly conserved.

Our studies showed that the polynucleotides and polypeptides of the invention are highly conserved at their 5' (N-terminal) ends and that there is a high degree of homology between BVH-3, BVH-11 and BVH-11-2 at the 5'-end. However, the 3'-ends of these genes are divergent and the homology between BVH-3 and BVH-11 or BVH-11-2 drops off at the 3-end.

We tested the full length polynucleotide and polypeptide and fragments thereof to determine whether they conferred immunity to virulent *Streptococcus* infection *in vivo*. The results are reported in the specification, specifically in Examples 5, 11 and 12. The results demonstrate that animals immunized with the full-length polypeptides or predicted mature polypeptides were protected from infection. We have also shown that antibodies specific to these polypeptides protect animals from deadly pneumococcal diseases such as sepsis and pneumonia. Moreover,

experimentation using a variety of different animal species including mouse, rabbit and monkeys indicates that the protection is not restricted to mice immune responses. These results support the usefulness of the claimed polynucleotides and polypeptides as vaccines in animals and humans.

Our studies with fragments of the BVH-3 and BVH-11 genes revealed the location of immunogenic epitopes within the full length molecules. (See Tables 6, 7, and 8). We discovered that epitopes conferring immunity are not located in the 5' (N-terminal) ends, but instead are located in the variable regions of these genes and polypeptides. We also discovered that chimeric genes encoding for proteins comprising protective epitopes from BVH-3 and BVH-11 (such as NEW12 protein described in Example 12) or BVH-11-2 appear more attractive than either single full-length or truncated protein based on immunological data.

Our studies using truncated gene products show that polypeptides containing the first 225 (BVH-3C) or 509 (BVH-3AD and L-BVH-3AD) or 353 (BVH-11A) amino acids of BVH-3 and BVH-11, respectively, do not confer immunity, while the region encoding the carboxyl end of the proteins was protective (See Tables 6, 7 and 8), thus allowing the identification of epitopes critical to protection-eliciting capacity in the carboxy two-thirds of BVH-3 and BVH-11. Altogether, our data from active and protection studies using animals vaccinated with BVH-3 truncates including BVH-3B, NEW1, NEW2, NEW3 and NEW15 or antibodies reactive with the latter confirmed the protective activity of immune responses to these BVH-3 gene fragments where protection was measured by either an increase in the survival rate or survival period for the vaccinated groups compared to the mock vaccinated animals.

We have data from active and passive protection studies that demonstrate the protective capacity of epitopes located on NEW1, NEW2, NEW3 and NEW15 epitopes. Active protection studies were performed in BALB/c mice as described in Example 11 in order to evaluate the protective capacity of BVH-3 gene products.

Table 1. Protection mediated by recombinant BVH-3 truncates^a

Immunogen	No. of mice alive : no. of mice dead 13 days post-challenge	Median day of death
BVH-3M	8 : 0	>13
NEW1	8 : 0	>13
NEW3	5 : 3	>13
BVH-15	5 : 3	>13
None	0 : 8	2

^a Vaccinated mice were challenged intravenously with virulent pneumococci.

Protection against experimental infection was observed in mice vaccinated with BVH-3M, NEW1, NEW3 or NEW15 when compared to the mice injected with the adjuvant alone. The table 2 illustrates the protection conferred by passive transfer of rabbit antibodies raised to BVH-3M molecule to CBA/N mice prior challenge with pneumococci.

Table 2. Protection mediated by rabbit antibodies raised to BVH-3M proteins in mice passive administered with antibody prior experimental infection with virulent *S. pneumoniae* WU2

Antibodies	Competitor	alive : dead ^a	Median days alive
Anti-BVH-3M	No competitor	5 : 0	>14
Anti-BVH-3M	NEW1	0 : 5	2
Anti-BVH-3M	NEW2	5 : 0	>14
Anti-BVH-3M	NEW3	5 : 0	>14
Anti-BVH-3M	NEW2 + NEW3	2 : 3	5
preimmune	No competitor	0 : 5	2
none	none	0 : 3	2

^a The number of mice alive versus the number of mice dead on day 14 post-challenge.

The incubation of rabbit BVH-3M antibodies with soluble antigen competitors to block the antigen binding sites of the antibodies was performed in order to establish the specificity of the protective antibodies. The observation that NEW1 abolished the protection clearly indicate the specificity of protective anti-BVH-3M antibodies for NEW1 epitopes. NEW2

(residues 472-800) and NEW3 (residues 800-1039) correspond to the first (amino end) and second half (carboxyl end) of NEW1 (residues 472-1039) molecule, respectively. Combination of NEW2 and NEW3 were required to significantly inhibit the protective anti-BVH-3M effect while no effect was observed when these molecules were used individually thus indicating that protective epitopes were present on both NEW1 subfragments, NEW2 and NEW3.

Similarly, we were able to show that BVH-11 truncates designated BVH-11B (also called NEW13), BVH-11C, NEW4, NEW5, NEW6, NEW8, NEW9 (see Example 11) and NEW16 provided protection against experimental disease and could be useful vaccine components.

Clearly, BVH-11 and BVH-11-2 constitute two distinct genes and gene products found in *S. pneumoniae*. The development of monoclonal antibodies to BVH-11-2 allowed us to demonstrate the existence of two different types of epitopes, shared or BVH-11-2-specific (see Example 9). BVH-11-2-specific reactive monoclonal antibodies were found to be directed against surface-exposed epitopes located on the very unique NEW11 molecule sequence. Protection data obtained with NEW10, NEW11, NEW14 and BVH-11-2M substantiate their use as vaccine components.

Our molecular and antigenic conservation studies revealed that the BVH-3 gene (SEQ ID NO. 1, 11) and BVH-11-2 gene (SEQ ID NO. 13) and/or protein (SEQ ID NO. 2 and SEQ ID NO. 14, respectively) were present in most, if not all of the 150 *S. pneumoniae* strains tested, independent of the capsular serotype. These observations made it clear that the proteins and fragments thereof have universal vaccine capability to prevent infection by any *S. pneumoniae* disease.

It is also my understanding that claims 16-20 and 25 are rejected under 35 U.S.C. § 102(a) over WO98/18930 as being anticipated. The Examiner states that the cited reference discloses an amino acid sequence that has 100% sequence similarity to SEQ ID NO. 2 over 447 amino acids and discloses two other sequences that share 78.6% sequence similarity with SEQ ID NO. 2 over 103 amino acids.

In regards to BVH-3, it is clear that prior to the filing of the present application, others had tried and failed to identify the full length gene. As a result, the cited reference, WO/18930, reports only partial sequences, which we have shown do not even encode polypeptide that confers immunity. It is noteworthy to mention that a stretch of 177 amino acid residues of the partial BVH-3 sequence reported by others is missing in some

pneumococcal isolates (SEQ ID NO 15 and 16). Vaccination of animals with recombinant BVH-3 molecules of strain SP63 (SEQ ID NO 16) which lack the amino acids corresponding to residues 244 to 420 on sequence of strain SP64 (SEQ ID NO 2) and BVH-3 of strain SP64 conferred equivalent protection against experimental pneumococcal infection, thus indicating that the portion absent in SP63 sequence was not essential for protection.

Furthermore, the cited prior art does not disclose the BVH-11-2 gene (SEQ ID NO. 13), which we isolated using the unique set of probes developed in our laboratory. As far as I know, the existence of BVH-11-2 had never been reported or suggested prior to the filing date of the present application. We have succeeded in isolating hybridomas that secrete antibodies reactive exclusively with BVH-11-2 molecules (see Table 4) and the development of a panel of specific immunological probes revealed that BVH-3, BVH-11 and BVH-11-2 genes were simultaneously expressed in *S. pneumoniae*. (see Examples 9 and 10)

Significant differences distinguish our claimed polynucleotides and polypeptides from those disclosed in WO 98/18930. For example, SEQ ID NO. 1, which encodes a 1039 amino acid residue gene product (SEQ ID NO. 2) (referred to herein as BVH-3) was not even known to exist prior to the present invention. To my knowledge, the polynucleotides and polypeptides corresponding to gene or protein regions responsible for protective immunity (e.g., SEQ ID NOs. 2, 10, 55, 60, 62, 67, 68, and 74) have never been predicted in the prior art.

Moreover, the prior art disclosure of similar polynucleotides does not disclose or suggest the location of the immunogenic epitopes within the BVH-11 polypeptides and therefore, does not suggest the use of BVH-11, BVH-11-2 or specifically, fragments thereof that contain these epitopes to confer immunity to any *S. pneumoniae* strain, regardless of serotype. WO 98/18930 merely discloses 127 polynucleotide sequences and polypeptide sequences without providing any distinction between conserved and non-conserved regions, or protective and non-protective regions.

The cited prior art also fails to disclose or suggest the molecular conservation of the genes and polypeptides of this invention throughout the *Streptococcus pneumoniae* species. Our discovery of the molecular homogeneity of these genes or gene fragments and their encoded polypeptides and the location of the immunogenic epitopes within the

polypeptides has enabled us to develop universal vaccines. These polypeptides and vaccines are clearly not anticipated by the cited prior art.

Finally, it is my belief that changes to the amino acid sequence of the claimed fragments of SEQ ID NO. 2 of 5% or less will not alter immunogenicity of the claimed fragments in most cases. This is particularly true since the skilled artisan would recognize that conservative amino acid changes might be made, and the data indicate that specific regions of the fragments are not essential for immunogenicity. Moreover, the amino acid sequence of BVH-3 of 33 different strains show that these polypeptides differ from one another at several sites. Thus, the claimed polypeptide fragments can tolerate up to 5% amino acid changes and still elicit an immune response. Further, testing for immune response is routine in the art.

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from SP64).
the alignment.

[illegible]

Figure 1
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Figure 1
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Figure 1

Table 3. Comparison of a variety of protein sequences to the SP64 sequence fragment corresponding to amino acid residues 408 to 1039 by pairwise alignment.

Strain designation	Scrogroup or type	Site of isolation	Source ^a	% DNA identity ^b	% protein identity ^b
SP11	34	NA	LSPQ	95	95
SP20	31	NA	LSPQ	97	97
SP35	10	Blood	LSPQ	98	98
SP40	7	Blood	LSPQ	96	96
SP44	16	Blood	LSPQ	98	98
SP46	18	Blood	LSPQ	97	97
SP55	9	NA	LSPQ	97	97
SP57	7	NA	LSPQ	96	96
SP63	9	Blood	LSPQ	97	96
SP70	9	Blood	LSPQ	99	99
SP71	1	Blood	LSPQ	98	98
SP74	23	Blood	LSPQ	99	99
SP79	5	NA	LSPQ	99	99
SP82	24	NA	LSPQ	98	98
SP93	6	Blood	CAREC	99	99
SP94	14	Blood	CAREC	99	99
SP96	19	Blood	CAREC	99	99
SP115	9V	NA	CHEO	96	96
SP134 ; WU2 ^c	3	NA	D. Briles	99	99
SP136 ; Rxl ^c	- ^a	Laboratory	D. Briles	99	99
SP138 ; BG9739 ^c	4	Blood	D. Briles	99	99
SP140 ; DBL6A ^c	6A	NA	D. Briles	98	98
SP141 ; P4241 ^c	3	Blood	M. Bergeron	98	98
SP142 ; DS2382-94 ^d	4	Blood	G. Carlone	99	98
SP145 ; DS2214-94 ^d	14	Blood	G. Carlone	98	98
SP147 ; DS2217-94 ^d	19F	Blood	G. Carlone	99	99
SP148 ; DS2216-94 ^d	23F	Blood	G. Carlone	98	99
SP149 ; EF5668 ¹⁰	4		D. Briles	99	99
SP150 ; EF6796 ^c	6A	Blood	D. Briles	99	99
SP151 ; BG7322 ^c	6B	Blood	D. Briles	99	99
SP152 ; JNR.7/87 ^{11,12}	4	NA	A. Camilli	99	99
SP153	19	Ear	LSPQ	94	94
SP154	23	Ear	LSPQ	99	99

^a The pneumococcal strains were from the collection of the Vaccine Research Unit, Centre de recherche du Centre Hospitalier de l'Université Laval, Sainte-Foy, Québec, Canada. The strains include isolates from 16 different serogroups or types (1, 3, 4, 5, 6, 7, 9, 10, 14, 16, 18, 19, 23, 24, 31 and 34). Strains were obtained from LSPQ ; Laboratoire de Santé Publique du Québec (Sainte-Anne-de-Bellevue, Qc Canada), CAREC ; Caribbean Epidemiology Centre (Port of Spain, Trinidad), CHEO ; Children's Hospital of Eastern Ontario (Ottawa, Canada), Dr. David Briles from the Department of Microbiology, University of Alabama at Birmingham (Birmingham, AL), Dr. Michel Bergeron from Centre de recherche en infectiologie, Centre

Hospitalier de l'Université Laval (Sainte-Foy, Qc, Canada), Dr. G. Carlone, Respiratory Diseases Immunology Section, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention (Atlanta, GA), and Dr. Andrew Camilli from the Department of Molecular Biology and Microbiology, Tufts University School of Medicine (Boston, MA).

^b % identity were calculated by pairwise ClustalW alignment of DNA or protein sequences using the MacVector software (Oxford Molecular Ltd.)

^c Rx-1 strain is a nonencapsulated derivative from type 2 strain D39.

5. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

7 June 2002
DATE

Josée Hamel
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Epitope Localization on BVH-3 Protein

